IJFANS
International Journal of Food And Nutritional Sciences

Volume 5, Issue 3, Jul-Sep 2016, www.ijfans.com e-ISSN: 2320-7876

# INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

**IMPACT FACTOR ~ 1.021**

**Official Journal of IIFANS**

IJFANS International Journal of Food And Nutritional Sciences

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

*e-ISSN* 2320 –7876 *www.ijfans.com*
Vol.5, Iss.3, Jul-sep, 2016


*Research Paper* *Open Access*

# FORMULATION AND EVALUATION OF MALTED INGREDIENT MIXES

**Shilpee Gupta and Jaswinder Brar**
Department of Food and Nutrition,

*Corresponding Author: shilpeeg21@gmail.com

**Received on: 14th April, 2015** **Accepted on: 2nd October, 2015**

## ABSTRACT

The study was aimed to formulate ingredient mixes with optimum amino acid composition using the process of malting and supplementation. Twenty-eight ingredient mixes were prepared from the malted key food ingredients such as cereals and legumes. The raw and germinated grains as well as malted ingredient mixes were evaluated for proximate composition, amino acids, *in vitro* protein digestibility (IVPD), *in vitro* starch digestibility (IVSD), *in vitro* iron availability and antinutrients. The processing of grains resulted in 6-19% increase in protein, 3-8% in lysine, 16-21% in IVPD, 14-25% in IVSD and 5-12% in *in vitro* iron availability while phytate and phenol were reduced by 22-43% and 23-50%, respectively as compared to their raw. There were negative correlation between *in vitro* digestibility and iron availability with anti-nutrients. Lysine content was increased significantly from 351 mg/100g in control to 535-897 mg/100g in formulated ingredient mixes. Similarly, a significant increase in IVPD was observed in the ingredient mixes, the value ranged from 71-82% against control i.e. 63%. Hence, it can be concluded that the nutritionally superior products with improved protein quality can be developed from such ingredient mixes specially for feeding vulnerable group (children, pregnant, lactating women and elderly).

Keywords: Malting, multigrains, lysine, *in vitro* protein digestibility, *in vitro* iron bioavailability, anti-nutrients.

## INTRODUCTION

Nowadays, people are greatly concerned not only about the quantity of food but also about nutritional quality. Dietary quality is an important limiting factor for a large lacto vegetarian population of India which depends on plant foods for obtaining adequate nutrition. The low bioavailability of nutrients, arising from the presence of antinutrients such as phytate, polyphenols, and oxalate, is another factor that limits the quality of predominantly plant based diets (Hotz and Gibson 2007, West *et al* 2002). Cereals constitute an important source of dietary calories and proteins in Indian diets, being good sources of carbohydrates, fibre, B-complex vitamins and minerals but limiting in lysine, an essential amino acid. Most legumes are rich in lysine but low in sulfur amino acids. Thus, combination of cereals and legumes appropriately complement each other with respect to their amino acid profile (Ihekoronye and Ngoddy 1985).

Legumes not only add variety to human diets but also serve as an economical source of supplementary proteins for majority of the vegetarian population (Sood *et al* 2002). However, the protein quality of foods depends not only on its amino acid composition but also on the availability of these amino acids. Hence, cereals and legumes and their products require specialized treatment in order to enhance their nutritional quality, organoleptic properties and shelf-life. Simple household food-processing approaches can be used to enhance the bioavailability of micronutrients in plant-based diets. These include thermal processing, mechanical processing, soaking, fermentation, and germination/malting. Germination is a complex metabolic process during which the lipids, carbohydrates and storage proteins within the seed are broken down in order to provide energy and amino acids necessary for the plant development (Zeigler 1995).

Germination of cereals and legumes has been shown to be generally advantageous as it improves their nutritional qualities (Corriea *et al* 2008). The enzymes of the resting grain gets activated during germination, resulting in conversion of cereal starch to fermentable sugars, partial hydrolysis of cereal proteins and other macromolecules. However, the effect of germination depends on the type of grain, the conditions and duration of the germination process (Savelkoul *et al* 1992). Germination increases protein and carbohydrates digestibility, enhances some of the vitamin contents, reduces the antinutritional factors and improves their overall nutritional quality (Malleshi and Klopfenstein 1996). It reduces the flatulence causative oligosaccharides and increases the concentration of amino acids (Udayasekhara and Belwady 1987).

Hence, the study was aimed to investigate the effect of malting and supplementation on the physicochemical accessibility of nutrients, reduction of antinutrient contents such as phytate, phenol or increase the content of compounds that improve bioavailability. With to increasing demand of protein supplements, the development of simple and cost effective processing options for developing countries in order to improve the protein quality of plant based products holds significance.

## MATERIALS AND METHODS

### PROCUREMENT OF CEREALS AND PULSES

The most common varieties of four cereals namely wheat (HD 2329), pearl millet (PCB 164), barley (PL 807) and oats (OL 9) and legumes namely mungbean (SML 668), bengal gram (PBG5), soybean (SL 744) and cowpea (C 367) were procured from the department of Plant Breeding and Genetics, PAU, Ludhiana and stored in air tight container for preparation of ingredient mixes.

### GERMINATION

The seeds were cleaned, washed and soaked in tap water in ratio of 1:3 (w/v) grains for 12 h at room temperature (32±2°C) and the water drained at each 4 h interval. At the end of the period, the seed were drained, spread separately and were allowed to germinate for 12, 24, 36, 48 and 60 h covered with damp cotton cloth to optimize most suitable time for germination for maximum nutrient availability and digestibility. Water was sprinkled at 12 h interval to facilitate the germination process. The seed samples were dried in hot air oven at 60 $^{o}$C till constant weight. The dried samples were ground into fine powder using stainless steel grinder and stored in air tight polythene bags for further analysis.

### FORMULATION OF INGREDIENT MIXES

Twenty-eight ingredient mixes were designed using selected malted cereals and legumes in different proportions in order to reach the chemical score of 100 by balancing limiting amino acid lysine in the wheat flour. The lysine content was used to calculate the chemical score as recommended by FAO/WHO/UNU (1985) using food composition tables of ICMR (2010). The formulations of each ingredient mix are shown in Table 1.

**Table 1 Formulation of ingredient mixes**

| Ingredient mixes | Flour Composition | Amount of key ingredients (per 100g) |
|---|---|---|
| **Control** | Whole wheat flour | 100 |
| **1** | Whole wheat flour+ Moong bean | 81+19 |
| **2** | Whole wheat flour+ Bajra flour+ Moong bean | 61+20+19 |
| **3** | Whole wheat flour+ Bajra flour+ Moong bean | 41+41+18 |
| **4** | Whole wheat flour+ Barley flour+ Moong bean | 62+21+17 |
| **5** | Whole wheat flour+ Barley flour+ Moong bean | 43+43+14 |
| **6** | Whole wheat flour+ Oat flour+ Moong bean | 62+21+17 |
| **7** | Whole wheat flour+ Oat flour+ Moong bean | 43+43+14 |
| **8** | Whole wheat flour+ Cow pea | 79+21 |
| **9** | Whole wheat flour+ Bajra flour+ Cow pea | 60+20+20 |
| **10** | Whole wheat flour+ Bajra flour+ Cow pea | 41+41+18 |
| **11** | Whole wheat flour+ Barley flour+ Cow pea | 62+21+17 |
| **12** | Whole wheat flour+Barley flour+Cow pea | 42+42+16 |
| **13** | Whole wheat flour+Oat flour+Cow pea | 61+20+19 |
| **14** | Whole wheat flour+Oat flour+Cow pea | 42+42+16 |
| **15** | Whole wheat flour+ Soybean | 77+23 |
| **16** | Whole wheat flour+Bajra flour+ Soybean | 59+20+21 |
| **17** | Whole wheat flour+Bajra flour+ Soybean | 40+40+20 |
| **18** | Whole wheat flour+Barley flour+ Soybean | 60+20+20 |
| **19** | Whole wheat flour+Barley flour+ Soybean | 42+42+16 |
| **20** | Whole wheat flour+Oat flour+ Soybean | 60+20+20 |
| **21** | Whole wheat flour+Oat flour+ Soybean | 42+42+16 |
| **22** | Whole wheat flour+ Bengal gram | 83+17 |
| **23** | Whole wheat flour +Bajra flour+ Bengal gram | 62+21+17 |
| **24** | Whole wheat flour+Bajra flour+ Bengal gram | 42+42+16 |
| **25** | Whole wheat flour+Barley flour+ Bengal gram | 64+21+15 |
| **26** | Whole wheat flour+Barley flour+ Bengal gram | 44+44+12 |
| **27** | Whole wheat flour+Oat flour+ Bengal gram | 64+21+15 |
| **28** | Whole wheat flour+Oat flour+ Bengal gram | 44+44+12 |

### CHEMICAL ANALYSIS

The raw and germinated cereals and legumes as well as malted ingredient mixes were subjected for chemical analysis. Standardized procedures of AOAC (2000) were followed to estimate moisture, total ash, crude fat, crude protein and crude fiber on dry matter basis while ascorbic acid was estimated in fresh samples in triplicates. Available carbohydrate was calculated by difference method. *In vitro* protein digestibility was estimated by Akeson and Stachmann (1964) method modified by Singh *et al* (1989). Amino acids *viz*. available lysine was determined by FDNB method of Carpenter (1960) modified by Booth (1971), methionine by Horn *et al* (1946) and cystine by Liddell and Saville (1959). *In vitro* starch digestibility was estimated by Singh *et al* (1982) method and *in vitro* iron availability by Rao and Prabhabati (1978) method. Anti nutritional factors *viz.* Phytin Phosphorus was determined by Haug and Lantzsch (1983) and Polyphenols by AOAC (2000).

### STATISTICAL ANALYSIS

The data obtained from chemical analysis was subjected to a one way analysis of variance (ANOVA) by using the statistical analysis software (SPSS) with a probability of P≤0.05 and Tukey's test was used. Mean and standard deviations were computed using MS Excel. The correlation coefficients were computed.

## RESULTS AND DISCUSSION

### NUTRITIONAL ANALYSIS OF RAW AND GERMINATED SAMPLES

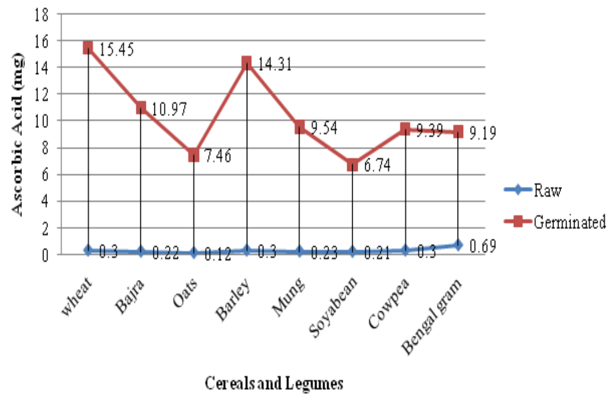
### PROXIMATE COMPOSITION

Table 2 shows the proximate composition of raw and germinated samples. Moisture content of raw seeds ranged from 8.72 to 11.79 g/100g. There was a reduction in moisture on germination in all samples. Similarly, a significant reduction in ash content was observed in all the samples after germination ranging from 7.02 to 28.48%. On the contrary, crude fibre contents increased significantly (p≤0.05) by 9.8-27.3% in all the samples. Leaching out of solid matter during pre-germination soaking might have resulted in reduction of mineral matter on germination (Ghavidel and Prakash 2007). Fat content of raw seeds ranged from 1.62-5.05 g/100g in cereals and 1.22-18.56 g/100g in legumes. Germination significantly decreased the fat content in all the samples. This agrees with report of Shah *et al* (2011) on mungbean varieties. Dhaliwal and Agrawal (1999) also reported that the fat content of soyabean decreased with germination time. However, Echendu *et al* (2009) found a significant increase of fat content after germination of ground beans. The decrease in fat content could be due to the use of fat as energy for developing sprouts. Protein content of raw samples ranged from 7.96-12.34 g/100g in cereals and 16.46-35.14 g/100g in legumes while the corresponding increased significantly (p<0.05) during germination ranged from 9.64-15.30 g/100g and 19.79-41.48 g/100g respectively. The percent increase being 6-19% in all the samples. Similar increases in protein have been reported for other legumes such as soybean (Kaushik *et al* 2010), lablab beans (Osman 2007) and mungbeans (Mubarak 2005). This increase in protein can be attributed to the utilization of carbohydrate as an energy source for sprouting (Donangelo *et al* 1995). There was a reduction in carbohydrate content in all the seed samples on germination ranging from 0.07 to 17.76%. The decrease might be due to α-amylase activity (Lasekan 1996). This α-amylase breaks down complex carbohydrates to simpler and more absorbable sugars which are utilized by growing seedlings during germination. Khalil *et al* (2007) while working on Kabuli and desi type chickpea varieties, reported significant increase in moisture, protein, ash content and decrease in nitrogen free extract (NFE) content as well as Inyang and Zakari (2008) also showed significant decrease in carbohydrate levels of the instant fura samples prepared using germinated and fermented pearl millet.

### ASCORBIC ACID

Vitamin C, which is practically absent in dry seeds increased in significant amounts after germination as shown in fig 1. The highest increase in ascorbic acid content from its initial value was found in wheat being 0.30 to 15.45 mg/100g among cereals and 0.23 to 9.54 mg/10g in mungbean among legumes. The percent increase being 98% in cereals and 92-98% in legumes. The results were comparable with findings of (Yang *et al* 2001) for germinated wheat seed. Riddoch *et al* (1998) reported that many species of pulses produced significant quantities of vitamin C up to five days following germination in both light and dark although cooking caused a marked loss of ascorbate. Likewise, significant increase in the content of ascorbic acid of different cereals and legumes seeds has also been reported by Harmuth-Hoene *et al* (1987).

**Fig 1 Effect of germination on ascorbic acid content of cereals and legumes per 100g fresh weight**




### AMINO ACIDS

The content of amino acids *viz.* lysine, methionine and cystine for cereal and legume samples are presented in Table 3. The increase in amino acids was highly significant ($p < 0.01$) in germinated samples. Available lysine, a limiting amino acid in cereals increased by 5-8% over raw on germination while for legumes, the increase was 3-8%. Methionine and cystine was increased by 3-14% and 2 to 5%, respectively in all the germinated samples. Most of the essential amino acids increased in quantity with germination. This could be due to the breakdown of complex polypeptides in the grain to simpler absorbable compounds (amino acids). This finding is in agreement with earlier reports of Yu-Haey *et al* (2004) and Hamad and Fields (1979) who reported appreciable increase in the levels of lysine in germinated wheat, barley, oats and rice. Echendu *et al* (2009) found a release of free amino acids after enzymatic hydrolysis for the synthesis of new protein.

**Table 2 Effect of germination on proximate composition of cereals and legumes (g/100g dry matter)**

| | | Moisture | Ash | Crude Fibre | Crude fat | Protein | Carbohydrate |
|---|---|---|---|---|---|---|---|
| **Wheat** | Raw | 11.79[a] ± 0.45 | 2.86[cd] ± 0.08 | 2.29[b] ± 0.34 | 1.62[d] ± 0.11 | 12.34[d] ± 0.25 | 69.10[a] ± 0.36 |
| | Germinated | 9.86[c] ± 0.03 | 2.50[de] ± 0.04 | 2.71[e] ± 0.35 | 1.42[d] ± 0.03 | 15.30[d] ± 0.38 | 68.21[a] ± 0.26 |
| | % Change | -19.60 | -13.75 | 15.25 | -14.01 | 19.38 | -1.32 |
| | t-value | 5.60* | 6.65** | 1.44 [NS] | 3.03* | 11.11** | 7.24** |
| **Bajra** | Raw | 11.57[ab] ± 0.73 | 2.13[d] ± 0.15 | 3.04[b] ± 0.06 | 5.05[c] ± 0.04 | 11.27[de] ± 0.38 | 66.94[a] ± 0.67 |
| | Germinated | 10.23[b] ± 0.11 | 1.99[ef] ± 0.20 | 3.48[d] ± 0.27 | 4.38[c] ± 0.12 | 13.11[de] ± 0.03 | 66.81[b] ± 0.35 |
| | % Change | -13.13 | -7.02 | 12.61 | -15.19 | 14.0 | - 0.19 |
| | t-value | 3.11* | 0.96 [NS] | 2.74 [NS] | 8.54** | 8.21** | 3.21* |
| **Oats** | Raw | 10.73[abc] ± 0.13 | 2.22[d] ± 0.15 | 7.67[a] ± 0.22 | 4.45[c] ± 0.17 | 7.96[e] ± 0.09 | 66.99[b] ± 0.57 |
| | Germinated | 9.44[d] ± 0.17 | 1.84[f] ± 014 | 8.83[a] ± 0.20 | 3.86[c] ± 0.18 | 9.64[f] ± 0.23 | 66.50[c] ± 0.96 |
| | % Change | -13.69 | -20.43 | 13.09 | -15.08 | 17.39 | - 2.54 |
| | t-value | 10.02** | 3.10* | 6.6 ** | 3.99* | 11.62** | 3.43* |
| **Barley** | Raw | 10.82[abc] ± 0.80 | 3.56[bc] ± 0.27 | 4.20[b] ± 0.51 | 1.84[d] ± 0.22 | 10.66[d] ± 0.93 | 68.92[a] ± 0.58 |
| | Germinated | 9.56[d] ± 0.07 | 2.77[cd] ± 0.50 | 5.74[b] ± 0.31 | 1.47[d] ± 0.37 | 11.60[ef] ± 0.55 | 68.86[a] ± 0.26 |
| | % Change | -13.17 | - 28.48 | 26.82 | -25.33 | 8.08 | -0.07 |
| | t-value | 2.71 [NS] | 2.39 [NS] | 4.39* | 1.49 [NS] | 1.49 [NS] | 5.02** |
| **Mung** | Raw | 9.09[bc] ± 1.32 | 4.01[ab] ± 0.17 | 4.25[b] ± 0.45 | 1.40[d] ± 0.43 | 22.5[b] ± 0.72 | 58.66[c] ± 1.69 |
| | Germinated | 7.25[e] ± 0.08 | 3.47[cd] ± 0.16 | 4.72[e] ± 0.211 | 1.04[d] ± 0.18 | 26.11[b] ± 1.50 | 57.41[e] ± 1.12 |
| | % Change | - 25.36 | -15.34 | 9.88 | - 34.18 | 13.48 | - 2.18 |
| | t-value | 2.40 [NS] | 3.90* | 1.60 [NS] | 1.29 [NS] | 3.66* | 1.96NS |
| **Soyabean** | Raw | 8.72[c] ± 0.65 | 4.83[a] ± 0.17 | 4.01[b] ± 0.27 | 18.56[a] ± 0.81 | 35.14[a] ± 1.25 | 28.74[d] ± 0.54 |
| | Germinated | 7.21[e] ± 0.03 | 4.42[a] ± 0.18 | 5.51[b] ± 0.10 | 16.96[a] ± 0.66 | 41.48[a] ± 2.67 | 24.42[f] ± 0.74 |
| | % Change | -21.03 | - 9.26 | 27.31 | - 9.39 | 15.29 | - 17 76 |
| | t-value | 4.02* | 2.87* | 8.78** | 2.62 [NS] | 3.71* | 3.05* |
| **Cowpea** | Raw | 11.32[abc] ± 1.93 | 3.87[abc] ± 0.94 | 3.73[b] ± 0.09 | 1.22[d] ± 0.19 | 23.08[b] ± 0.77 | 56.78[c] ± 2.55 |
| | Germinated | 10.75[a] ± 0.07 | 3.60[b] ± 0.12 | 4.52[c] ± 0.10 | 0.91[d] ± 0.05 | 24.57[b] ± 0.33 | 55.65[e] ± 0.38 |
| | % Change | -5.30 | - 7.58 | 17.52 | -33.81 | 6.06 | - 2.03 |
| | t-value | 0.51 [NS] | 0.49[NS] | 9.74** | 2.62 [NS] | 3.04* | 0.81 [NS] |
| **Bengal gram** | Raw | 9.47[abc] ± 0.30 | 3.35[bc] ± 0.52 | 4.06[b] ± 0.32 | 5.60[b] ± 0.14 | 16.46[c] ± 0.92 | 61.06[b] ± 0.47 |
| | Germinated | 7.17[e] ± 0.03 | 3.28[bc] ± 0.38 | 5.55[b] ± 0.28 | 5.16[b] ± 0.42 | 19.79[c] ± 1.22 | 59.05[d] ± 0.29 |
| | % Change | -32.12 | - 2.33 | 26.87 | - 8.58 | 16.80 | - 3.38 |
| | t-value | 13.05** | 0.20 [NS] | 6.00** | 1.72 [NS] | 3.74* | 6.52** |

Values are presented as Mean ± SD. [a-c] Means followed with different superscripts are significantly (P<0.05) different using tukey's test. ** Significant at 1% level of significance, *Significant at 5% level of significance, NS-Non significant

**Table 3 Effect of germination on Lysine, Methionine and Cystine content of cereals and legumes (mg/100g dry weight)**

| | Lysine (mg) | | | | Methionine (mg) | | | | Cystine (mg) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Raw** | **Germinated** | **% change** | **t-value** | **Raw** | **Germinated** | **% change** | **t-value** | **Raw** | **Germinated** | **% change** | **t-value** |
| **Wheat** | 323.7$^{f}$ ± 2.13 | 351.6$^{f}$ ± 2.88 | 7.93 | 13.45** | 183.3$^{de}$ ± 14.43 | 196.6$^{a}$ ± 7.63 | 6.77 | 3.06* | 261.5$^{c}$ ± 2.65 | 268.6$^{c}$ ± 1.15 | 2.65 | 4.26* |
| **Bajra** | 351.1$^{e}$ ± 1.96 | 373.3$^{e}$ ± 2.88 | 5.94 | 11.01** | 268$^{c}$ ± 15.71 | 283.3$^{c}$ ± 14.43 | 5.41 | 1.24 $^{NS}$ | 201.4$^{f}$ ± 2.65 | 210.7$^{f}$ ± 1.15 | 4.33 | 5.46** |
| **Barley** | 348.3$^{e}$ ± 2.88 | 369$^{e}$ ± 3.60 | 5.60 | 7.75** | 165.5$^{de}$ ± 24.66 | 191.7$^{a}$ ± 14.43 | 13.65 | 4.27* | 183.3$^{h}$ ± 1.15 | 188.5$^{g}$ ± 1.15 | 2.82 | 5.65** |
| **Oats** | 353.3$^{e}$ ± 2.88 | 371.5$^{e}$ ± 2.88 | 4.93 | 7.77** | 181.3$^{e}$ ± 2.30 | 196.6$^{a}$ ± 5.77 | 7.79 | 0.65 $^{NS}$ | 179.2$^{g}$ ± 1.15 | 186.7$^{g}$ ± 2.30 | 3.92 | 4.91** |
| **Mung** | 1993.2$^{b}$ ± 5.77 | 2058.3$^{b}$ ± 14.43 | 3.15 | 7.24** | 319.1$^{b}$ ± 16.18 | 337.5$^{b}$ ± 33.07 | 5.46 | 0.86 $^{NS}$ | 230.1$^{d}$ ± 0.23 | 241.8$^{d}$ ± 2.88 | 4.77 | 6.89** |
| **Soyabean** | 2765$^{a}$ ± 1.73 | 3003.2$^{a}$ ± 2.88 | 7.93 | 122.62** | 500.9$^{a}$ ± 44.91 | 516.7$^{a}$ ± 14.43 | 3.04 | 0.57 $^{NS}$ | 690.3$^{a}$ ± 0.57 | 707.3$^{a}$ ± 4.61 | 2.40 | 6.32** |
| **Cowpea** | 1659.9$^{d}$ ± 0.11 | 1723.1$^{d}$ ± 2.88 | 3.67 | 38.01** | 332.4$^{cd}$ ± 12.93 | 358.2$^{c}$ ± 14.43 | 7.21 | 2.31 $^{NS}$ | 309.6$^{c}$ ± 0.69 | 321.5$^{e}$ ± 2.88 | 3.75 | 7.07** |
| **Bengal gram** | 1203.5$^{c}$ ± 3.07 | 1271.6$^{c}$ ± 2.88 | 5.35 | 27.98** | 231.4$^{b}$ ± 16.36 | 250$^{b}$ ± 0 | 7.44 | 1.96 $^{NS}$ | 219.7$^{b}$ ± 0.46 | 231.9$^{b}$ ± 2.88 | 5.15 | 7.04** |

Values are presented as Mean ± SD.
$^{a-c}$ Means followed with different superscripts are significantly (P<0.05) different using tukey's test.
** Significant at 1% level of significance, *Significant at 5% level of significance, NS-Non significant

**Table 4 Effect of germination on *invitro* protein and *invitro* starch digestibility of cereals and legumes**

| **Cereals and Legumes** | ***Invitro* protein digestibility (%)** | | | | ***Invitro* starch digestibility (%)** | | | |
|---|---|---|---|---|---|---|---|---|
| | Raw | Germinated | % change | **t-value** | Raw | Germinated | % change | **t-value** |
| **Wheat** | 63.5$^{b}$ ± 2.22 | 80.5$^{bc}$ ± 1.65 | 21.12 | **10.60**** | 68.6$^{ab}$ ± 1.15 | 85.0$^{a}$ ± 5.03 | 18.88 | **5.36**** |
| **Bajra** | 54.5$^{c}$ ± 2.05 | 68.4$^{e}$ ± 1.59 | 20.25 | **9.22**** | 65.0$^{ab}$ ± 1.73 | 75.9$^{ab}$ ± 4.04 | 14.17 | **4.20*** |
| **Barley** | 62.4$^{b}$ ± 0.60 | 77.3$^{c}$ ± 1.46 | 19.32 | **16.33**** | 62.5$^{b}$ ± 2.51 | 83.0$^{a}$ ± 7.54 | 24.69 | **4.49*** |
| **Oats** | 45.5$^{d}$ ± 1.94 | 56.2$^{f}$ ± 2.03 | 18.90 | **6.42**** | 55.3$^{c}$ ± 5.03 | 67.7$^{b}$ ± 2.51 | 18.27 | **3.79*** |
| **Mung** | 71.3$^{a}$ ± 0.62 | 86.6$^{a}$ ± 1.15 | 17.72 | **20.18**** | 66.4$^{ab}$ ± 2.16 | 87.4$^{a}$ ± 3.07 | 23.84 | **9.34**** |
| **Soyabean** | 61.0$^{b}$ ± 6.14 | 73.1$^{d}$ ± 0.94 | 16.43 | **3.34*** | 63.0$^{ab}$ ± 2.64 | 79.3$^{ab}$ ± 10.0 | 20.63 | **2.71$^{NS}$** |
| **Cowpea** | 66.4$^{a}$ ± 0.72 | 79.1$^{bc}$ ± 1.30 | 15.96 | **14.61**** | 66.7$^{a}$ ± 2.30 | 84.3$^{a}$ ± 4.04 | 16.64 | **5,09**** |
| **Bengal gram** | 67.3$^{a}$ ± 1.57 | 82.4$^{b}$ ± 0.74 | 18.21 | **14.91**** | 67.6$^{ab}$ ± 2.68 | 88.0$^{a}$ ± 3.46 | 23.22 | **8.22**** |

Values are presented as Mean ± SD.
$^{a-c}$ Means followed with different superscripts are significantly (P<0.05) different using tukey's test. ** Significant at 1% level of significance, *Significant at 5% level of significance, NS-Non significant

**Table 5 Effect of germination on *invitro* iron availability of cereals and legumes**

| | Total iron (mg) | | | | Ionizable iron (mg) | | | | *In vitro* Iron availability (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Raw | Germinated | % change | t-value | Raw | Germinated | % change | t-value | Raw | Germinated | % change | t-value |
| **Wheat** | 5.06[d] ± 0.25 | 4.76[d] ± 0.37 | -6.29 | 1.14 NS | 0.36[f] ± 0.02 | 0.37[f] ± 0.02 | 1.01 | 0.19 NS | 3.65[cd] ± 0.35 | 4.15[c] ± 0.06 | 12.11 | 4.22* |
| **Bajra** | 7.50[c] ± 0.30 | 6.90[c] ± 0.26 | -8.69 | 2.59 NS | 0.56[e] ± 0.02 | 0.55[e] ± 0.03 | -2.94 | 0.64 NS | 4.05[cd] ± 0.08 | 4.25[c] ± 0.08 | 4.64 | 2.79* |
| **Barley** | 1.69[e] ± 0.29 | 1.55[e] ± 0.25 | -9.46 | 0.65 NS | 0.09[g] ± 0.01 | 0.11[g] ± 0.01 | 15.48 | 1.15 NS | 3.20[d] ± 0.24 | 3.65[d] ± 0.35 | 12.31 | 4.79** |
| **Oats** | 3.20[f] ± 0.20 | 2.90[f] ± 0.10 | -10.34 | 2.32 NS | 0.15[g] ± 0.20 | 0.15[g] ± 0.03 | -3.28 | 0.36 NS | 2.87[c] ± 0.14 | 3.02[c] ± 0.19 | 5.17 | 0.89 NS |
| **Mung** | 4.90[d] ± 0.45 | 4.56[d] ± 0.40 | -7.29 | 0.94 NS | 0.83[d] ± 0.07 | 0.82[d] ± 0.09 | -0.58 | 0.06 NS | 8.41 [b] ± 0.06 | 8.92[b] ± 0.28 | 5.78 | 3.07* |
| **Soyabean** | 10.01[a] ± 0.34 | 9.18[a] ± 0.57 | -9.07 | 2.17 NS | 1.80[a] ± 0.11 | 1.75[a] ± 0.11 | -3.10 | 0.58 NS | 8.96[b] ± 0.26 | 9.46[b] ± 0.03 | 5.25 | 3.23* |
| **Cowpea** | 8.76[d] ± 0.47 | 7.85[d] ± 0.22 | -11.58 | 3.02* | 1.58[c] ± 0.08 | 1.49[c] ± 0.03 | -6.17 | 1.81 NS | 9.02[a] ± 0.20 | 9.45[a] ± 0.05 | 4.58 | 3.58* |
| **Bengal gram** | 5.14[b] ± 0.51 | 4.74[b] ± 0.50 | -8.57 | 0.98 NS | 1.15[b] ± 0.12 | 1.14[b] ± 0.12 | -0.88 | 0.09 NS | 11.06[b] ± 0.74 | 11.87[b] ± 0.78 | 6.84 | 1.30NS |

Values are means ± SD of three independent replicates.
[a-c] Means followed with different superscripts are significantly (P<0.05) different using tukey's test.
*Significant at 5% level of significance, NS-Non significant, *In vitro* available iron based on prediction equation for iron absorption using percent ionizable iron at pH 7.5 was calculated: $Y= 0.4827+0.4707X$, where X is percent ionizable iron at pH 7.5.

**Table 6 Effect of germination on anti nutrients of cereals and legumes (mg/100g dry weight)**

| Cereals and Legumes | Phytate (mg) | | | | Phenol (mg) | | | |
|---|---|---|---|---|---|---|---|---|
| | Raw | Germinated | % change | t-value | Raw | Germinated | % change | t-value |
| **Wheat** | 234.6[a] ± 3.05 | 176.6[a] ± 2.88 | - 32.83 | 23.90** | 381.3[e] ± 0.75 | 292.0[c] ± 2.64 | -30.59 | 56.21** |
| **Bajra** | 139.0[d] ± 1.73 | 100.5[d] ± 2.30 | -38.07 | 23.00** | 305.5[f] ± 0.50 | 223.7[d] ± 0.63 | -36.56 | 175.02** |
| **Barley** | 98.3[e] ± 2.51 | 70.0[d] ± 2.00 | -40.47 | 15.26** | 433.3[c] ± 1.20 | 351.8[a] ± 10.30 | -23.22 | 75.00** |
| **Oats** | 124.6[f] ± 1.15 | 102.3[e] ± 0.57 | -21.82 | 29.96** | 227.8[h] ± 0.81 | 175.2[e] ± 0.89 | -30.04 | 13.60** |
| **Mung** | 141.3[d] ± 0.57 | 98.6[d] ± 1.15 | -43.24 | 57.29** | 396.6[d] ± 1.60 | 292.8[c] ± 2.56 | -35.44 | 59.43** |
| **Soyabean** | 233.2[a] ± 1.15 | 179.3[a] ± 1.15 | -30.11 | 57.27** | 440.7[b] ± 1.28 | 344.4[a] ± 0.52 | -28 | 120.46** |
| **Cowpea** | 185.4[c] ± 0.57 | 130.0[c] ± 9.64 | -42.56 | 9.92** | 254.5[g] ± 1.48 | 182.5[e] ± 0.83 | -39.41 | 130.25** |
| **Bengal gram** | 155.1[b] ± 2.30 | 113.4[b] ± 1.15 | -37.05 | 70.00** | 481.1[a] ± 1.78 | 321.3[b] ± 1.15 | -49.74 | 73.20** |

Values are presented as Mean ± SD.
[a-c] Means followed with different superscripts are significantly (P<0.05) different using tukey's test.
** Significant at 1% level of significance, *Significant at 5% level of significance

## IN VITRO PROTEIN AND STARCH DIGESTIBILITY (IVPD AND IVSD)

Table 4 shows percentage protein and starch digestibility of germinated cereals and legumes compared to the raw. Germination appreciably improved the IVPD by 16 to 21% and IVSD by 14 to 25% as compared to control. Wheat had the highest IVPD among cereals while mungbean had the highest IVPD among legumes. These findings were in agreement with work by other similar research (Archana *et al* 2001, Trugo *et al* 2000 and Preet and Punia 2000).

## IN VITRO IRON AVAILABILITY

Table 5 reveals the in vitro iron availability of cereals and legumes. Iron content of raw samples ranged from 1.69 to 7.50 mg/100g in cereals and 4.9 to 10.10 mg/100g in legumes. Decrease in iron content was content was observed on germination which could be due to leaching of solid matter during pre-germination soaking. However, the percent bioavailability of iron increased significantly by 5-12% in cereals and 5-7% in legumes on germination. The results are in line with report of (Giri *et al* 1981). The food processing methods such as germination and malting have been found to enhance iron absorption due to elevated vitamin C content or reduced tannin or phytic acid content, or both (Tontisirin *et al* 2002).

## ANTINUTRITIONAL COMPONENTS

Table 6 presents the antinutritional components of cereals and legumes. Phytate content in raw samples ranged from 98.3 mg/100g in barley to 234.6 mg/100g in wheat while phenol content ranged from 254.5 mg/100g in cowpea to 433.3mg/100g in barley. There were marked reduction in phytate and phenol on germination of cereal and legume samples studied. The 21.82 to 43.24% decrease in phytate and 23.22 to 49.74% decrease in phenol were observed after germination. These decreases may be attributed to the increased activity of phytase and polyphenol oxidase and other catabolic enzymes as observed by (Kruger 1976) for wheat. However, according to Goupy *et al* (2003), an increase of 50% for phenolic compounds was observed during the germination of barley grains followed by kilning.

## RELATIONSHIP BETWEEN ANTI-NUTRIENTS AND *IN VITRO* PROTEIN AND STARCH DIGESTIBILITY AND IRON AVAILABILITY

Antinutrients viz. phytate and phenols were significantly and negatively correlated with *in vitro* protein digestibility (r = -0.43 and -0.56), *in vitro* starch digestibility (r = -0.52 and -0.24), *in vitro* iron availability (r = -0.43 and -0.41), iron (r = -0.65 and -0.54) and Vitamin C (r = -0.16 and -0.54) as shown in table 7. The results were in line with the reported studies. However, Vitamin C did not show any significant relationship with phytate and *in vitro* starch digestibility with phenol. Phytic acid had a significant ($P<0.05$) negative correlation with digestibility (*in vitro*) of both starch and protein of rabadi (Gupta and Sehgal 1991).

**Table 7 Corelation coefficients of anti-nutrients with *in vitro* protein and starch digestibility and iron availability**

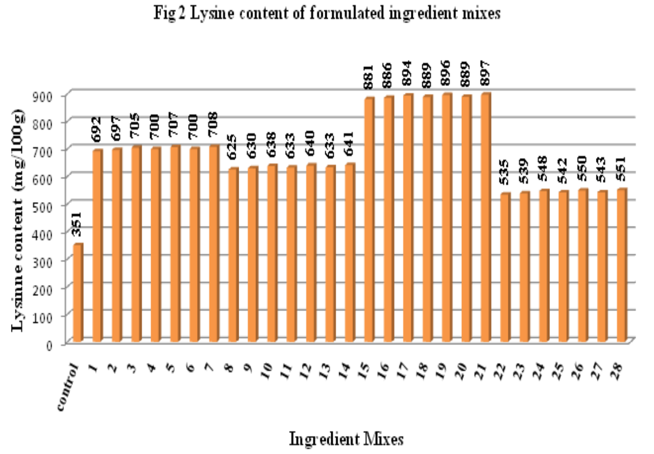
| | Phytate | Phenol |
|---|---|---|
| ***In vitro* protein digestibility** | - 0.43* | -0.56* |
| ***In vitro* starch digestibility** | - 0.52** | $-0.24^{NS}$ |
| ***In vitro* iron bioavailability** | - 0.43* | - 0.41* |
| **Iron** | - 0.65** | -0.54** |
| **Vitamin C** | $-0.16^{NS}$ | - 0.54** |

## NUTRITIONAL ANALYSIS OF FORMULATED INGREDIENT MIXES

## LYSINE CONTENT

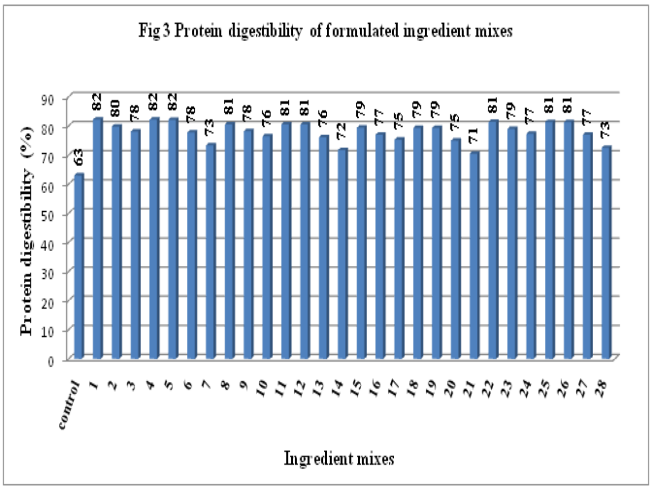
Fig 2 reveals the lysine content of whole wheat flour as control and the formulated ingredient mixes. Lysine content of control was found to be 351 mg/100g. A significant improvement was observed in lysine content of all the ingredient mixes. It was expected due to supplementation of legumes. Highest lysine content was found in soybean supplemented mixes ranged from 881-897 mg/100g followed by mungbean supplemented mixes ranged from 692-702 mg/100g and cowpea supplemented mixes ranged from 625-641 mg/100g.

Fig 2 Lysine content of formulated ingredient mixes




## *IN VITRO* PROTEIN DIGESTIBILITY (IVPD)

Effect of malting and supplementation on IVPD of formulated ingredient mixes was shown in fig 3. IVPD of the formulated ingredient mixes was found to be higher than control, the value ranged from 71-82% as compared to control i.e. 63%. The results were in agreement with the study of Anigo *et al* 2010 who reported 75-82% of IVPD in complementary formulated using malted cereals and soybean.




## CONCLUSION

Germination improved the nutritional worth of cereals and legumes by enhancing the bioavailability and digestibility of nutrients and reducing the antinutrients. The contents of protein, amino acids, ascorbic acid, in vitro iron availability and in vitro protein and starch digestibility improved significantly while ash, carbohydrate and iron content decreased on germination. Phytates and phenols reduced by 22 - 43% and 23 - 50% respectively on germinated samples over control. Supplementation of malted cereals and legumes in different proportions improved the protein quality of formulated ingredient mixes with optimum amino acid composition. Hence, the formulated ingredient mixes can be further used to prepare various products like *chapati*, biscuits, bread, noodles, extruded snacks etc. and can be used for feeding vulnerable group (children, pregnant, lactating women and elderly) having nutritionally better and easily digestible forms of sugars and amino acids.

## REFERENCES


- Akeson W E and Stachman M A (1964) A pepsin-pancreatin digest index of protein quality evaluation. *J Nutr* 83:257-61.
- Anigo K M, Ameh D A, Ibrahim S and Danbauchi S S (2010) Nutrient composition of complementary food gruels formulated from malted cereals, soybeans and groundnut for use in North-western Nigeria. *Afr J Food Sci* 4(3):65-72.
- AOAC (2000) Official methods of analysis. Association of official Analytical Chemists, 16th ed. Washington, USA.
- Archana, Sehgal S and Kawatra A (2001) In vitro protein and starch digestibility of pearl millet (*Pennisetum gluacum* L.) as affected by processing techniques. *Nahrung Food* 45(1):25-7.
- Booth V H (1971) Problems in determination of FDNB – Available lysine in animal protein foods. *J Sci Food Agric* 22:658-66.
- Carpenter K J (1960) The estimation of available lysine in Animal Protein foods. *Biochem J* 77:604-10.
- Correia I, Nunes A, Barros A S and Delgadillo I (2008) Protein profile and malt activities during sorghum germination. *J Sci Food Agric* 88:2598-605.
- Dhaliwal Y S and Agrawal R A (1999) Composition of fat in soyabeans as affected by duration of germination and drying temperature. *J Food Sci Technol* 36:266-67.
- Donangelo C M, Trugo L C, Trugo N M F and Eggum B O (1995) Effect of germination of legume seeds on chemical composition and on protein and energy utilization in rats. *Food Chem* 53(1):23-7.
- Echendu C A, Obizoba I C and Anyika J U (2009) Effects of germination on chemical composition of groundbean (Kerstingiella geocarpa harm) seeds. *Pak J Nutr* 8:1849-54.
- FAO/WHO/UNU (1985) *Energy and protein requirements.* Report of a joint FAO/WHO/UNU Expert Consultation. WHO Tech Rep Ser 724. WHO, Geneva.
- Ghavidel R A and Prakash J (2007) The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds, *LWT* 40(7):1292-99.
- Giri J, Pravatham R and Santhini K (1981) Effect of germination on the levels of pectin, phytins and minerals in the selected legumes. *Indian J Nutr Diet* 18:87-91.
- Goupy P, Dufour C Loonis M and Dangles O (2003) Quantitative kinetic analysis of hydrogen transfer reactions from dietary polyphenols to the DPPH radical, *J Agric Food Chem* 51(3):615-22.
- Gupta C and Sehgal S (1991) Development, acceptability and nutritional value of weaning mixtures. *Plant Food Hum Nutr* 41:107-17.
- Hamad A M and Fields M L (1979) Evaluation of the protein quality and available lysine of germinated and fermented cereal. *J Food Sci* 44(2):456-59.
- Harmuth-Hoene A E, Bognar A E, Kornemann U and Diehl J F (1987) The influence of germination on the nutritional value of wheat, mung beans and chickpeas. *Z Lebensm Unters Forsch* 185(5):386-93.
- Haug W and Lantzsch H J (1983) Sensitive method for the rapid determination of phytates in cereals and cereal products. *J Sci Food Agric* 34:1423-27.
- Horn M J, Jones D B and Blum A E (1946) Colorimetric determination of methionine in proteins and foods. *J Biol Chem* 166:313-20.
- Hotz C and Gibson R S (2007) Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. *J Nutr* 137:1097-1100.
- ICMR (2010) *Dietary guidelines for Indians*. A manual of National Institute of Nutrition.
- Ihekoronye A I and Ngoddy P O (1985) *Integrated food science and technology for the tropics*. Macmillan Publishers, London.

- Inyang C U and Zakari U M (2008) Effect of germination and fermentation of pearl millet of proximate chemical and sensory properties of instant Fura- a Nigerian Cereal Food. *Pakistan J Nutr* 7(1):9-12.
- Kaushik G, Satya S and Naik S N (2010). Effect of domestic processing techniques on the nutritional quality of the soybean. *Mediterr J Nutr Metab* 3(1):39-46.
- Khalil AW, Zeb A, Mahmood F, Tariq S, Khattak A B and Shah H (2007) Impact of germination time on comparative sprout quality characteristics of desi and Kabuli type chickpea cultivars (*Cicer arietinum* L). *LWT* 103:115-20.
- Kruger J E (1976) Changes in the polyphenol oxidase of wheat during kernel growth and maturation. *Cereal Chem* 53:201-11.
- Lasekan O O (1996) Effect of germination on alpha-amylase activities and rheological properties of sorghum (*Sorghum biocolar*) and acha (*Digitaria exilis)* grains. *J Food Sci Technol* 33:329-31.
- Liddell H P and Saville B (1959) Colorimetric determination of cystine. *Analyst* 84:188-90.
- Malleshi N G and Klopfenstein C F (1996) Proximate composition, amino acid and vitamin contents of malted chickpea, mungbean and their seed coats. *J Food Sci Technol* 33:479-82.
- Mubarak A E (2005) Nutritional composition and antinutritional factors of mung bean seeds (Phaseolus aureus) as affected by some home traditional processes. *Food Chem* 89:489-95.
- Osman M A (2007). Effect of different processing methods, on nutrient composition, antinutritional factors, and in vitro protein digestibility of Dolichos Lablab bean (Lablab purpuresus L) sweet. *Pakistan J Nutr* 6 (4): 299-303.
- Preet K and Punia D (2000) Proximate composition, Phytic acid, Polyphenols and digestibility (in vitro) of four brown cowpea varieties. *Int J Food Sci Nutr* 51:189-93.
- Rao B S N and Prabhavati T (1978) An *in vitro* method for predicting the bioavailability of iron from foods. *Am J Clin Nutr* 31:169-75.
- Riddoch C H, Mills C F and Duthie G G (1998). An evaluation of germinating beans as a source of vitamin C in refugee foods. *Eur J Clin Nutr* 52:115-18.
- Savelkoul F H, Vander poel A F and Tamminga S (1992) The presence and inactivation of trypsin inhibitors, tannins, lectins and amylase inhibitors in legume seeds during germination-A review. *Plant Food Human Nutr* 42:71-85.
- Shah S A, Aurang Zeb, Tariq M, Nadia N, Abbas S J, Samiullah M, Alim M A and Asim M (2011) Effect of sprouting time on biochemical and nutritional qualities of mungbean varieties. *Afr J Agric Res* 6(22):5091-98.
- Singh U, Kherdekor M S and Jambunathan R (1982) Studies on desi and kabuli chickpea cultivars the levels of amylase inhibitors, level of oligosaccharides and *in vitro* starch digestibility. *J Food Sci Technol* 27:82-85.
- Singh U, Rao P V, Seetha R and Jambunathan R (1989) Nutrient loss due to scarification of pigeonpea (Canjanus cajan L.) cotyhledons. *J Fd Sci* 54:974-81.
- Sood M, Malhotra S R and Sood B C (2002) Effect of processing and cooking on proximate composition of chickpea (Cicer arientinum) varieties. *J Food Sci Technol* 39:69-71.
- Tontisirin K, Mantel G and Battacharjee L (2002). Food based strategies to meet the challenges of micronutrient malnutrition in the developing world. *Proc Nutr Soc* 61:243-50.
- Trugo L C, Donangele C M, Trugo N M and Bach Knudsen K E (2000) Effect of heat treatment on nutritional quality of germinated seeds. *J Agric Food Chem* 48:2082-86.
- Udayasekhara R and Belwady B (1987) Oligosaccharides in pulse, varietal differences and effects of cooking and germination. *J Agric Food Chem* 26:316-19.
- West C W, Eilander A and Van Lieshout M (2002) Consequences of revised estimates of carotenoid bioefficacy for dietary control of vitamin A deficiency in developing countries. *J Nutr* 132(9):2920S–6S.
- Yang F, Basu T K and Ooraikul B (2001) Studies on germination conditions and antioxidant contents of wheat grain. *Int J Food Sci Nutr* 52(4):319-30.
- Yu-Haey K, Pascale R, Fernand L, Juana F and Vidal-Valverde C (2004) Effects of different germination conditions on the contents of free protein and non-protein amino acids of commercial legumes. *Food Chem* 86(4):537-45.
- Ziegler P (1995) *Carbohydrate degradation during germination*. In: Kigel J, Galili G (eds) Seed development and germination. Marcel Dekker Inc, New York, pp 447-74.